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Evaluation of an Expanded Two-ELISA Approach for Confirmation of Reactive Serum Samples in an HIV-Screening Programme for Pregnant Women

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Serum specimens were collected from 31,232 pregnant women in Amsterdam between 1988 and 1995 in a screening programme for human deficiency virus (HIV) infection. The sera of 56 (0.179%) women tested were confirmed as positive for HIV. A total of 67 sera reacted positive or borderline by the screening enzyme-linked immunosorbent assay (ELISA) and indeterminate or negative by HIV-1 Western blot; 42 of these specimens were available for evaluation of the strategy for diagnosis of HIV infection. A two-ELISA approach with the second ELISA based on a principle different from that of the screening ELISA, expanded with the use of a membrane immunoassay based on two synthetic peptides specific for HIV-1 gp41 and HIV-2 gp36 envelope proteins, was compared with the Western blot analysis. Indeterminate results were resolved with a nucleic acid sequence-based amplification assay (NASBA) for HIV-1 RNA and a strip immunoassay (SIA) for the simultaneous detection of antibodies to HIV-1 or HIV-2 and HIV-1 p24 antigen. Eleven samples were weakly or borderline positive by the screening test and gave indeterminate results by Western blot. The expanded two-ELISA approach designated these sera as HIV-negative, and confirmed negative by NASBA and the SIA. Twenty-one samples showed borderline or positive results on the screening test and negative results by Western blot. Again, these sera were characterised as HIV-negative by the expanded two-ELISA procedure, and this characterisation was confirmed by both NASBA and the SIA. Five HIV-2-positive serum samples were recognised by the expanded two-ELISA approach and the SIA; these sera were negative by NASBA. Finally, another five serum samples were weakly or borderline positive by both ELISAs and positive by the membrane immunoassay; of these five, two sera generated positive patterns and the other three indeterminate patterns on Western blots, and four were positive by the NASBA assay. Follow-up serum specimens from these five women were negative and the reactivity of the initial specimens was thus likely to have been the result of cross-contamination. Our results demonstrate the effectiveness of a simple confirmation approach of two HIV ELISAs expanded with a membrane spot assay to discriminate between infection with HIV-1 or HIV-2. The data also indicate the importance of retesting individuals with indeterminate or positive confirmational results to exclude the possibility of contamination as the cause of reactivity of the original specimen. ***J. Med. Virol. 54:285–290, 1998.***

KEY WORDS: HIV-confirmation; expanded-2-ELISA approach; HIV-infection in pregnancy

INTRODUCTION

The possible spread of human immunodeficiency virus (HIV) infection among the heterosexual population in Amsterdam has been monitored since 1988 by a screening programme targeted on pregnant women. Any sera found reactive by the HIV screening enzyme-linked immunosorbent assay (ELISA) were investigated further and tested by a confirmatory procedure that included repeating the initial test and performing a second ELISA based on a different principle. In some instances, an HIV-1 and HIV-2 synthetic peptide-specific assay and an HIV-1 p24 assay were carried out. The Western blot assay was used as a gold standard to confirm the presence of HIV-1 or HIV-2 antibodies [CDC, 1989,1991]. False positive or borderline ELISA results and indeterminate Western blot results are problematic for the diagnosis of HIV infection, espe-

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cially in a low-prevalence population. As described by Mortimer et al. [1992] and Gürtler [1996], the confirmation of HIV-screening ELISA reactive sera can be undertaken by the two-ELISA approach. We have now evaluated in retrospect the confirmatory strategy of the Amsterdam screening programme and the use of the two-ELISA approach expanded with a membrane immunoassay based on two synthetic peptides specific for HIV-1 gp41 and HIV-2 gp36 envelope proteins. The HIV-1 RNA nucleic acid sequence-based amplification assay (NASBA), and a recently developed strip immunoassay (SIA) were used to resolve indeterminate results of the HIV confirmatory testing procedure.

MATERIALS AND METHODS

Study Population

From 1988, pregnant women who attended either obstetric clinics for antenatal care or midwife practices in Amsterdam were invited to participate in the HIV screening programme. Blood specimens were collected during the first 3 months of pregnancy for routine prenatal examination (blood group and rhesus factor, syphilis serology, and hepatitis B surface antigen). The women participated on a voluntary basis and their sera were tested with informed consent. The women were interviewed about HIV risk factors. In 1989, the programme was extended to an abortion clinic and three outpatient infertility clinics in Amsterdam [Bindels et al., 1994]. Of the eligible pregnant women, ≅93% participated. Between 70% and 75% of all pregnant women in the city were reached by this programme in 1990 and 1991. Because of the low prevalence of infection, from 1991 surveillance was restricted to two sentinel hospitals and one midwife practice [Bindels et al., 1996]. Up to 1995, a total of 31,232 women participated in the screening programme; the sera of 56 (0.179%) of these women initially found positive and were subsequently confirmed positive for HIV.

Sera Included in the Study

A total of 67 sera collected from 1988 to 1995 that gave positive or borderline results by ELISA and indeterminate results or negative results by Western blot was eligible for this study, 42 of which were still available for analysis. Fifteen serum samples that were positive by both the screening ELISA and the confirmational procedure, and 10 ELISA-negative sera were randomly selected as positive and negative controls, respectively.

HIV Serology

Screening assays. Different screening assays were used over the course of the programme. From 1988 to June 1989, Abbott Recombinant HTLV-III EIA (Abbott, North Chicago, IL); July 1989 to June 1994, Abbott Recombinant HIV1/HIV2 EIA, (Abbott, Wiesbaden-Delkenheim, Germany); June 1994 to June 1995: Abbott Recombinant HIV1/HIV2 EIA, 3rd generation (Abbott, Wiesbaden-Delkenheim, Germany); and from July 1995 Abbot Third generation Plus EIA with HIV-1 O-antigen (Abbott, Wiesbaden-Delkenheim, West Germany). Each of these assays is based on the double-antigen principle. The absorbance values were interpreted according to the instructions of the manufacturer. Samples with an optical density cutoff ratio (OD/CO) of < 2.0 and > 0.5 were considered to be borderline positive.

Retesting of reactive samples. All reactive samples were retested by the initial screening assay and by a second ELISA based on a different principle: Wellcozyme HIV Recombinant (HIV1/HTLVIII/LAV1; Wellcome Diagnostics, Dartford, England) or from July 1992 Murex Diagnostics Benelux, Utrecht, The Netherlands). This test is based on a competitive principle and is specific for the detection of antibodies to HIV-1.

Western blot. Initially, the Biotech/Dupont HIV-1 Western Blot (Biotech/Dupont, Wilmington, DE) was used, followed from 1989 by the HIV-1/-2 Western Immunoblot (Diagnostic Biotechnology, Singapore). The results of the latter tests were interpreted according to the instructions of the manufacturer. The absence of virus-specific bands was interpreted as a negative result; the presence of at least one band from each of the envelope (gp41, gp120, or gp160), core antigen (p17, p24, or p55), and the endonuclease-polymerase (p31, p51, or p66) was interpreted as a positive result for antibodies to HIV-1, and the presence of an HIV-2-specific band could reflect a positive or indeterminate result for HIV-1, with HIV-2 confirmation indicated. All other patterns that did not meet the criteria for HIV-1 positivity or indication for HIV-2 confirmation were interpreted as indeterminate.

Additional tests. These included the polyclonal and monoclonal HIV Ag-1 (Abbott Laboratories, North Chicago, IL). Both assays are based on the same indirect sandwich ELISA principle, but differ in the use of polyclonal or monoclonal antibodies to HIV-1, respectively.

The Pepti-LAV 1-2 (Diagnostics Pasteur, Marnes-la-Coquette, France) assay was used if HIV-2 confirmation was indicated. For the present study, all available sera were tested by this assay which is based on a membrane spotted with two synthetic peptides specific for HIV-1 gp41 and HIV-2 gp36 envelope proteins. Sera reactive with the HIV-2 spot were tested with an HIV-2 Western blot (Liatek, Organon Teknika, Oss, The Netherlands, or Diagnostic Biotechnology, Singapore) in the Laboratory of Virology of the National Institute of Health and Protection of the Environment in Bilthoven, The Netherlands (R. van den Akker).

Tests carried out in retrospect on stored sera. Sera were stored at –20°C and tested in retrospect by more recently available assays.

The Deciscan HIV Ag-Ab (Sanofi Diagnostics Pasteur, Marnes la Coquette, France) is an enzyme immunoassay in strip format (SIA) for the simultaneous detection of antibodies to HIV-1 or HIV-2 and HIV-1 antigen (p25). The test incorporates a recombinant protein and peptides derived from HIV-1, peptides spe-

TABLE I. Serum Samples, Obtained From 11 Pregnant Women, Which Reacted (weakly) Positive, or Borderline by an Abbott Screening Test but Were Indeterminate by Western Blot

Number	S/CO[a] Abbott	Western blot	CO/S[a] Wellcozyme	NASBA	Deciscan p-env-1[b]	Deciscan r-env-1[c]	Deciscan HIV-1 Ag
1	1.47	(p55)[d]	0.52	Neg	Neg	Neg	Neg
2	1.16	(p55)	0.49	Neg	Neg	Neg	Neg
3	1.62	(p55 p53) gp160	0.05	Neg	Neg	Neg	Neg
4	2.29	(gp120) gp160	0.63	Neg	Neg	Neg	Neg
5	1.24	(gp160)	0.52	Neg	Neg	Neg	Neg
6	5.25	(p15 p24 gp160)	0.65	Neg	Neg	Neg	Neg
7	5.36	p15 (p24 gp41 gp160)	0.52	Neg	Neg	Neg	Neg
8	5.41	p15 p24 (gp160)	0.30	Neg	Neg	Neg	Neg
9	1.11	(p55)	0.64	Neg	Neg	Neg	Neg
10	0.23	(gp41)	0.44	Neg	Neg	Neg	Neg
11	1.21	(p55 gp120 gp160)	0.49	Neg	Neg	(Pos)	Neg

[a]Optical density sample/cutoff ratio for the Abbott test and the Wellcozyme assay, respectively.
[b]HIV-1 peptide.
[c]HIV-1 recombinant.
[d]Between parentheses: weak.

cific for HIV-2, and a monoclonal antibody to HIV-1 p24.

Finally, the qualitative HIV-1 RNA NASBA (Organon Teknika, Turnhout, Belgium) which detects HIV-1 RNA in serum samples, was also carried out in the present study. This assay is based on primer-dependent isothermal amplification and is able to amplify target RNA in a direct and selective manner as result of simultaneous activities of avian myeloblastosis virus reverse transcriptase (AMV-RT), ribonuclease H, and T7RNA polymerase [Compton, 1991; Kievits et al., 1991]. RNA was extracted from 100-μl serum samples as described by Boom et al. [1990]. The primers comprised the gag 1 primer set described by Van Gemen et al. [1994]. Amplicons were detected by specific hybridisation with a horseradish peroxidase-labelled oligonucleotide. Hybridised material was separated from free probe by polyacrylamide gel electrophoresis and direct staining of the bands in the gel was achieved by precipating tetramethylbenzidine substrate. The qualitative NASBA assay is able to detect HIV-1 RNA below 100 copies per sample [Gobbers et al., 1997; Van Damme et al., 1995].

Definition of Positive, Indeterminate, and Negative Samples

A sample was considered positive when it tested positive repeatedly on both the screening assay and the second ELISA, and generated a positive Western blot pattern according to the criteria described above. A serum sample was considered negative when the screening assay was negative or when a weak initial ELISA reactivity could not be confirmed by the second ELISA and Western blot. A serum sample was considered indeterminate when the screening assay was repeatedly borderline or positive, the second ELISA test was negative or borderline, and an incomplete HIV-1 Western blot pattern was observed. A sample was considered positive for antibodies to HIV-2, when a HIV-2 Western blot was positive.

According to the two-ELISA approach, a serum specimen was considered HIV-1-positive when both the screening ELISA and the second ELISA were positive, and the Pepti-LAV HIV-1 band was positive. The two-ELISA approach designated a specimen HIV-2-positive when the screening ELISA was positive, positivity of the second ELISA was not required, and the Pepti-LAV HIV-2 band was positive [Who, 1996].

Data Analysis

Data are presented as means standard deviation (S.D.) and were analysed with CIA [Gardner et al., 1991] and Epi Info 6.3 [Dean et al., 1994].

RESULTS

HIV Serology

Results of testing control sera. The 15 positive control sera gave positive results in all additional HIV-1 tests used. The mean sample/cutoff (S/CO) ratios for the Abbott test and Welcozyme were 7.88 (S.D. + 2.97) and 11.60 (S.D. + 7.18), respectively. All negative control sera but one were negative by the assays; the mean S/CO ratios for the Abbott test and Welcozyme were 0.15 (SD + 0.01) and 0.47 (S.D. + 0.07), respectively. The exception was obtained from a woman who was positive previously in screening assays. Her present serum was negative by both the Abbott third generation Plus and the Wellcozyme HIV recombinant ELISA, but it yielded a gp160 band on Western blot. The serum from this individual was negative by NASBA and the other additional tests.

Results of testing sera from study participants. On the basis of Western blot results, the sera of the study participants were divided into four categories:

(i) Eleven samples were weakly or borderline positive by the screening ELISA, negative by the second ELISA, and indeterminate by Western blot as shown in Table I. The mean S/CO ratios for the Abbott test and Welcozyme were 2.40 (S.D. + 1.95) and 0.48 (S.D. + 0.17, respectively. All samples in this category tested negative for Pepti-LAV, NASBA, and Deciscan test. These sera were considered negative by the two-ELISA

TABLE II. Serum Samples Obtained From Five HIV-2-Positive Pregnant Women

Number, HIV-1 Ag	S/CO[a] Abbott	Western blot	CO/S Wellcozyme	NASBA	Deciscan p-env-1[b]	Deciscan r-env-1[c]	Deciscan p-env-2[d]
33, Neg	2.75	(p24)[e] p31 (p53 p64 gp160)	0.64	Neg	(Pos)	(Pos)	Pos
34,Neg	8.06	p24 p55 p31 p53 (p64) gp41	0.57	Neg	Neg	Neg	Pos
35,Neg	6.20	p15 p24 p55 p31 p53 gp41 (gp120 gp41)	0.71	Neg	(Pos)	(Pos)	Pos
36,Neg	4.62	p15 p24 p55 p31 p53 (p64 gp160)	0.99	Neg	Neg	Neg	Pos
37,Neg	16.0	p24 (p31 gp160)	1.03	Neg	(Pos)	(Pos)	Pos

[a]Optical density sample/cutoff ratio for the Abbott test and the Wellcozyme assay, respectively.
[b]HIV-1 peptide.
[c]HIV-1 recombinant.
[d]HIV-2 peptide.
[e]Between parentheses: weakly positive or negative.

approach. The Western blot assay yielded bands that could explain the ELISA reactivity of these sera.

(ii) Twenty-one samples were borderline (n = 15) or positive (n = 6) on the screening ELISA, negative by the second ELISA, and negative by Western blot. The mean S/CO ratios for the Abbott test and Welcozyme were 1.63 (S.D. + 0.59) and 0.52 (S.D. + 0.01), respectively. All sera in this category were negative by the Pepti-LAV. According to two-ELISA approach these sera were considered negative. The sera were negative by the NASBA, whereas one yielded a faint reactive HIV-2 env band by the Deciscan.

(iii) Five samples tested positive on the screening ELISA and negative or borderline on the Wellcozyme assay. The results are presented in Table II. The mean S/CO ratios for the Abbott and Welcozyme ELISAs were 7.53 (S.D. + 5.13) and 0.79 (S.D. + 0.21), respectively. These five sera were positive for HIV-2 gp36 on the Pepti-LAV and were considered HIV-2- positive according the two-ELISA approach. The HIV-1 Western blot patterns of these samples either met the requirements for HIV-1 positivity or were indeterminate. All five sera were positive by the HIV-2 Western blot, positive for the HIV-2 env band by the Deciscan test, and negative for HIV-1 RNA by NASBA.

(iv) Five sera obtained in 1991, four of which were tested in two pairs consecutively, were (weakly or borderline) positive by both the screening and the second ELISA (Table III). The mean S/CO ratios for the Abbott and Welcozyme ELISAs were 2.54 (S.D. + 1.16) and 1.92 (S.D. + 0.87), respectively. Two sera generated positive patterns and the other gave indeterminate results by Western blot analysis. All five samples were positive for HIV-1 by the Pepti-LAV assay, and were HIV-1-positive according the two-ELISA approach. The Deciscan test was positive for HIV-1, and four of the five sera were positive by NASBA. However, these women did not belong to any high risk group for HIV infection and second specimens from each were negative by the screening ELISA, the second ELISA, and by Western blot. The original samples were therefore assumed to be contaminated by other HIV-positive serum specimens.

Results of Testing Follow-Up Serum Specimens

Second serum samples were obtained from 7 of the 11 participants in category (i). Whereas the first specimen from one participant (subject 1) showed a faint band against p55 by Western blot analysis, the second sample did not generate any band. The second serum samples from subjects 3, 6, and 7 showed the same band pattern by Western blot analysis as did the first samples. The follow-up specimens from subjects 4 and 5, who showed solitary envelope protein bands by Western blots on initial analysis, were again positive by the screening assay and reacted with the envelope bands by Western blots. Finally, the second serum sample from subject 11 was negative by both the screening ELISA and Western blot.

Follow-up serum samples were obtained from only 4 of the 21 women in category (ii). All four follow-up samples again were negative by Western blot.

The second serum samples obtained from the HIV-2-positive women in category (iii) again tested positive for HIV-2, and, as mentioned above, the follow-up specimens from the women in category (iv) were all negative.

DISCUSSION

The strategy advocated by Mortimer et al. [1992] and more recently by Gürtler [1996] as the two-ELISA approach was evaluated by the present study. Because we detected several HIV-2- positive women, this approach was extended with the Pepti-LAV assay which differentiates between HIV-1 and HIV-2 infection [Gnann et al., 1987]. The results of this expanded ELISA approach indicated that 32 (76%) of the 42 sera that were (weakly) positive or borderline by the screening ELISA could be considered as negative for HIV-1 and HIV-2. Eleven of these 32 sera generated bands on Western blots that might explain this ELISA reactivity, whereas 21 sera were negative by Western blot. Five (12%) of the 42 study participants were recognised as HIV-2- positive; sera from these women showed a dissociation in the OD/CO ratios by the Abbott screening ELISA and the HIV-1-specific Wellcozyme assay. An-

TABLE III. Serum Samples, Obtained From Five Pregnant Women, Which Reacted Positive by the Abbott Screening Test and Wellcozyme Test; Subsequent Serum Samples Reacted Negative

Number	S/CO[a] Abbott	Western blot	CO/S Wellcozyme	NASBA	Deciscan p-env-1[b]	Deciscan r-env-1[c]	Deciscan HIV-1 Ag
38	1.14	p24 (p31)[d] gp160	1.05	Pos	Pos	Pos	Neg
39	2.09	p24 (p55 p31 p53 p64 gp120) gp160	1.31	Pos	Pos	Pos	Neg
40	3.71	gp120 gp160	2.77	Pos	Pos	Pos	Neg
41	3.23	gp120 gp160	2.57	Pos	Pos	Pos	Neg
42	2.52	(gp120) gp160	2.04	Pos	Pos	Pos	Neg

[a]Optical density sample/cutoff ratio for the Abbott test and the Wellcozyme assay, respectively.
[b]HIV-1 peptide.
[c]HIV-1 recombinant.
[d]Between parentheses: weak.

other 5 (12%) of the 42 serum samples that were HIV-1-positive by the extended ELISA approach, appeared to be positive as a result of cross-contamination. Thus, it was concluded that incorporating the HIV-1- and HIV-2- specific peptide test in a simple confirmational procedure was beneficial for endorsing a negative confirmation result and confirming possible cases of HIV-2 infection. In a previous study, it was shown that the Pepti-LAV spot test detected all 18 known cases of HIV-2 infection in The Netherlands that were traced retrospectively [Van Doornum et al., 1991].

The NASBA procedure was used in this study as an additional test to resolve borderline ELISA and indeterminate Western blot results. A clear distinction was apparent between the specimens that were considered to be HIV-1-positive or negative on the basis of the confirmational procedure followed. The negative results by NASBA could be influenced by the long storage of the specimens. However, HIV-1 RNA in blood samples is fairly stable even when clinical samples undergo multiple cycles of freezing and thawing [Shirasaka et al., 1996]. The utility of the NASBA for resolution of indeterminate confirmatory results might be improved by including primer combinations that can detect all HIV-1 subtypes and HIV-2 with a high sensitivity.

No discrepancies were apparent between the results obtained with the Deciscan assay and those obtained with the confirmational strategy of the expanded two-ELISA approach. We thus conclude that, in the format used, this test is also of value for identifying HIV-1- or HIV-2- positive samples.

In contrast to the indeterminate Western blot patterns apparent with sera obtained from blood donors without evidence of HIV infection [Brooks Jackson et al., 1990], we could not establish a pattern specific for pregnant women [Brooks Jackson et al., 1990]. The only trend recognised was the presence of Env bands on the blots of 8 of the 11 participants in category (i). Indeterminate Western blot patterns are observed repeatedly in healthy individuals as well as in patients with autoimmune disorders [Kämmerer et al., 1995]. Low-risk blood donors can also show false-positive results by Western blot. The trend towards increased detection of Env only or p24 and Env only on Western blots appears related to the enhanced sensitivity of more recent enzyme immunoassays that recognise antibodies to gp41 or p24 antibodies [Sayre et al., 1996].

The importance of the request for a second serum sample has been demonstrated by the five women whose first specimens were confirmed positive for HIV-1 infection as a result of cross-contamination. Caution was warranted because the women did not belong to a high-risk group for HIV infection and because of the relatively low OD/CO ratios obtained in the screening test and the second ELISA. Therefore, the women were not informed that their sera were reactive when the second specimen was collected. Several explanations were considered for the probable contamination of the samples. Carry-over by the probe of the pipetting instrument did not appear responsible because the original samples were also reactive on the other assays performed manually. Mislabelling of the specimens was not a plausible explanation because the results of other tests, such as blood group and rhesus factor performed with the follow-up specimens, corresponded with those of the first specimens. We believe the most likely explanation for the reactivity of the first sera was their contamination with HIV- positive samples during handling. Handling of the sera could not be traced to one single technician. However, after a thorough review of laboratory procedures and safety regulations, we have not experienced further cases of possible mishandling of serum specimens. It was found that transfer of much less than 20 μl of an HIV-1-positive specimen to 2 ml volume of a negative sample is sufficient to result in a positive ELISA test for the latter.

It is concluded that the results demonstrate the usefulness of a simple strategy for confirming the reactivity of serum samples in an HIV screening ELISA by testing with a second ELISA based on a different principle and with a membrane spot test based on specific HIV-1 and HIV-2 peptides. The use of a test to detect the presence of antibodies to HIV-1 or HIV-2 as well as HIV-1 antigen was also of value in confirming serum samples reactive by the screening ELISA. Furthermore, nucleic acid amplification-based tests such as the NASBA procedure can identify definitively HIV-infected or uninfected individuals. Our data also indi-

cate the importance of retesting individuals with indeterminate or confirmation results.

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REFERENCES

Bindels PJE, Mulder-Folkerts DKF, Boer K, Schutte MF, Vander Velde WJ, Wong F, Van den Hoek JAR, Van Doornum GJJ, Coutinho RA. (1994): The HIV-prevalence among pregnant women in the Amsterdam region (1988-1991). European Journal of Epidemiology 10:331–338.

Bindels PJE, Mulder-Folkerts DKF, Schutte MF, Smit-van Wijk I, Boer K, Coutinho RA (1996): Resultaten van de screening op HIV-antistoffen bij zwangere vrouwen in de Amsterdamse peilstations, 1988-1995. Nederlands Tijdschrift voor Geneeskunde 140:2296–2298

Boom R, Sol CJA, Salimans MMM, Jansen CL, Wertheim-Van Dillen PME, Van der Noordaa. (1990) Rapid and simple method for purification of nucleic acids. Journal of Clinical Microbiology 28:495–503.

Brooks Jackson J, MacDonald KL, Cadwell J, Sullivan C, Kline WE, Hanson M, Sannerud KJ, Stramer SL, Fildes NJ, Kwok SY, Sninsky JJ, Bowman RJ, Polesky HF, Balfour HH, Osterholm MT (1990): Absence of HIV infection in blood donors with indeterminate western blot tests for antibody to HIV-1. New England Journal of Medicine 322:217–222.

Centers for Disease Control. (1989): Interpretation and use of the Western blot assay for serodiagnosis of human immunodeficiency virus type 1 infections. Morbidity and Mortality Weekly Reports 38/S-7:1–7.

Centers for Disease Control. (1991): Interpretive criteria used to report Western blot results for HIV-1. Morbidity and Mortality Weekly Reports 40:692–695.

Compton J (1991): Nucleic acid sequence-based amplification. Nature 350:91–92.

Dean AG, Dean JA, Coulombier D, Brendel KA, Smith DC, Burton AH, Dicker RC, Sullivan K, Fagan RF, Arner TG (1994): "Epi Info, Version 6: A word processing, databases, and statistics program for epidemiology on microcomputers." Atlanta, GA: Centers for Disease Control and Prevention.

Gardner MJ, Gardner SB, Winter PD (1991): Confidence interval analysis. British Medical Journal

Gnann JW, McCormick JB, Mitchell S, Nelson JA, Oldstone MB (1987) Synthetic peptide immunoassay distinguishes HIV type 1 and HIV type 2 infections. Science 237:1346–1349.

Gobbers E, Fransen K, Oosterlaken T, Janssens W, Hendrickx L, Ivens T, Vereecken K, Van de Wiel P, Van der Groen G (1997) Reactivity and amplification efficiency of the NASBA HIV-1 RNA amplification system with regard to different HIV-1 subtypes. Journal of Virological Methods 66(2):293–301.

Gürtler L (1996): Difficulties and strategies of HIV diagnosis. Lancet 348:176–179.

Kämmerer R, Bürgisser P, Frei PC (1995): Anti-human immunodeficiency virus type 1 antibodies of noninfected subjects are not related to autoantibodies occurring in systemic disease. Clinical and Diagnostic Laboratory Immunology 2:458–461.

Kievits T, Van Gemen B, Van Strijp D, Schukkink R, Dircks M, Adriaanse H, Malek L, Sooknanan R, Lens P (1991): NASBA isothermal enzymatic in vitro nucleic acid amplification optimized for the diagnosis of HIV 1 infection. Journal of Virological Methods 35: 273–286.

Mortimer J (1992): An alternative approach to confirming anti-HIV reactivity: A multi-country collaborative study. Bulletin WHO 70: 751–756.

Sayre KR, Dodd RY, Tegtmeier G, Layug L, Alexander SS, Busch MP. 1996. False positive human immunodeficiency virus type 1 Western blot tests in noninfected blood donors. Transfusion 36:45–52.

Shirasaka T, Kojima F, Mitsuya H. (1996): Stability of HIV-1 RNA in blood samples from patients with HIV-1 infection as determined by a quantitative polymerase chain reaction-based assay. Clinical and Diagnostic Virology 7:121–124.

Vandamme AM, Van Dooren S, Kok W, Goubau P, Fransen K, Kievits T, Schmit JC, De Clercq E, Desmyter J (1995): Detection of HIV1 RNA in plasma and serum samples using the NASBA amplification system compared to RNA-PCR. Journal of Virological Methods 52:121–132.

Van Gemen B, Van Beuningen R, Nabbe A, Van Strijp D, Jurriaans S, Lens P, Kievits T (1994): A one-tube quantitative HIV 1 RNA in plasma using nucleic acid amplification assay using electrochemiluminiscent (ECL) labelled probes. Journal of Virological Methods 49:157–168.

Van Doornum GJJ, Van den Akker R, Buitenwerf J, Kroes ACM, Coutinho RA, Lelie PN (1991): HIV-2 in Nederland. Nederlands Tijdschrift voor Geneeskunde 135:2129–2133.

WHO (1990a): Proposed WHO criteria for interpreting results from Western blot assays for HIV-1, HIV-2, and HTLV-I/HTLV-II. Weekly Epidemiological Records 1990:281-283.

WHO (1990b): Recommendations for the interpretation of HIV-2 Western blot results. Weekly Epidemiological Records 1990:74–75.